The Use of the R6 Transgenic Mouse Models of Huntington's Disease in Attempts to Develop Novel Therapeutic Strategies

Jia Yi Li, Natalija Popovic, and Patrik Brundin

Neuronal Survival Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, BMC A10, SE-221 84 Lund, Sweden

Summary: Huntington's disease (HD) is a genetic neurodegenerative disorder. Since identification of the disease-causing gene in 1993, a number of genetically modified animal models of HD have been generated. The first transgenic mouse models, R6/1 and R6/2 lines, were established 8 years ago. The R6/2 mice have been the best characterized and the most widely used model to study pathogenesis of HD and therapeutic interventions. In the present review, we especially focus on the characteristics of R6 transgenic mouse models and, in greater detail, describe the different therapeutic strategies that have been tested in these mice. We also, at the end, critically assess the relevance of the HD mouse models compared with the human disease and discuss how they can be best used in the future.
Key Words: Huntington's disease, therapy, transgenic mice, R6/2, neurodegenerative diseases.

INTRODUCTION

Huntington's disease (HD) is a genetic neurodegenerative disorder in which the mutation has been known for over a decade, but there is still not effective treatment. In 1996, the first transgenic mouse models of HD, named the R6/1 and R6/2 lines, were developed.[1] These models have been followed by many new HD transgenic lines of mice that differ regarding the type of mutation expressed, portion of the protein included in the transgene, promoter employed and level of expression of the mutant protein.[2] Despite the wealth of different transgenic HD mice available, the R6/1 and R6/2 lines have remained the most used models when testing novel therapies for HD.

The purpose of this review is to briefly describe the pathology of HD, the characteristics of the R6 transgenic mouse models and, in greater detail, the different therapeutic strategies that have been tested in these mice. In addition, we critically assess the relevance of these HD mouse models to the human disease and discuss how they can be best used in the future.

Address correspondence and reprint requests to Jia Yi Li, Neuronal Survival Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, BMC A10, SE-221 84 Lund, Sweden. E-mail: Jia-Yi.Li@med.lu.se.

WHAT IS HUNTINGTON'S DISEASE?

HD is an autosomal dominant disorder with a prevalence of around 1 in 10000, involving motor, cognitive, and psychiatric symptoms. The typical features include hyperkinetic involuntary movements, progressive dementia and personality changes that may include aggressiveness and paranoid psychosis. The *HD* gene codes for a protein named huntingtin, which has multiple functions that are still not fully understood. The gene is located on the short arm of chromosome 4 and when mutated it exhibits an expansion in the number of CAG trinucleotide repeats in the exon 1 of the gene.[3] Normal individuals have 35 or fewer CAG repeats in this locus and HD gene carriers contain 36 or more CAG repeats.[4] The expanded CAG repeat gives rise to an abnormally long polyglutamine stretch in the mutant huntingtin. This, as is discussed in greater detail below, causes the protein to misfold and to acquire toxic properties. The age of onset of symptoms is inversely related to the number of CAG repeats. Thus, individuals with around 40–55 CAG repeats typically develop symptoms around 35–45 years of age, whereas when the repeat expansion is in excess of 70 or more the onset of disease can be juvenile.[4] Typically, patients live 15–20 years from the onset of the first clear symptoms, and then die of complications due to immobilization such as aspiration pneumonia, urinary tract infections or sequel to pressure sores. In classical

descriptions, the neuropathology in HD is focused on the basal ganglia and neocortex. The most marked neuronal loss occurs in the caudate nucleus and putamen, as well as layers III, IV, and VI in the cerebral cortex.[4] A few studies have also described that the hypothalamus is afflicted with clear neuronal loss occurring in the lateral tuberal nucleus.[5,6] Aside from causing cell death, misfolded huntingtin also accumulates in the cytoplasm and nucleus leading to the formation of aggregates. These inclusions also contain several other proteins including components of the ubiquitin-proteasome pathway, chaperones, synaptic proteins, and transcription factors.[7,8] In patients who have had an adult onset of HD symptoms, around 3–6% of cortical neurons exhibit inclusions when examining the brains after death. Although this is a relatively small proportion of the cells, it should be pointed out that over 50% of the striatal neurons and around 20% of the cortical neurons have already died by that time, and those displaying inclusions at the time when the patient passes away could simply be a population of cells that are about to die. It has also been suggested that the cells exhibiting protein aggregates have actively evaded death by sequestering the mutant protein and that this is the reason that they remain alive.[9] Therefore, the idea that inclusions are protective against the toxic effects of expanded polyglutamine proteins has emerged.[9,10] However, even in case the protein aggregates allow the cells to survive longer, it is not clear to what extent they lead to disturbed cell function. Regardless of the ultimate effect of the protein inclusions, they are probably an important marker of the disease process in HD patients. Taken together, it is clear that two features of the human disease would be valuable to mimic in mouse models of HD, namely neuronal death in selected brain regions and the formation of intranuclear and cytoplasmic aggregates containing the mutant protein with an extended polyglutamine stretch.

MOUSE MODELS OF HD

The R6/1 and R6/2 transgenic mice were the first transgenic mouse models developed to study HD. They both express *exon* 1 of the human *HD* gene with around 115 and 150 CAG repeats, respectively.[1] The transgene expression in those mice is driven by the human huntingtin promoter. The resulting levels of transgene expression are around 31% and 75% of the endogenous huntingtin in the R6/1 and R6/2 models, respectively. After the generation of the R6/1 and R6/2 mice, numerous other transgenic mouse models of HD have been developed.[2] They vary concerning several parameters making each of them unique and therefore making it difficult to compare studies conducted in different mouse models. One crucial variable that differs between models is the length of the CAG repeat that is expressed. In addition, another important difference is the size of the fragment, in most cases only a fragment of the whole huntingtin protein is expressed. The R6 mice only express *exon* 1 (out of a total of 67 exons in the whole gene) coding for only about 3% of the N-terminal region of the protein, which includes the polyglutamine stretch.[1] In contrast, other models express larger portions of huntingtin, up to the full-length protein in some cases.[11] The promoter driving the transgene expression is also an important factor influencing the expression level of mutant protein and thereby the development of pathology. Some of the mice are knock-in models of HD,[2] which means that the CAG repeat is expressed in the mouse homolog of huntingtin and that the expression of mutant huntingtin is controlled by the endogenous mouse promoter. Naturally, the background strain onto which the transgenic mice are bred is also of vital importance because there are many modifier genes that can influence the *HD* gene and these are likely to differ between mouse strains.[2] Out of all the existing mouse models of HD, the R6/2 mouse is one that develops symptoms the most rapidly and has the most widespread occurrence of huntingtin inclusions in the brain. There are several reviews devoted to descriptions and comparisons of the different transgenic mouse models of HD,[2,12,13] and we will not go deeper into the subject here. Instead, we have chosen to focus our attention on the R6 lines of mice because they are not only the first to have been developed, but they are the most widely used in therapeutic trials and have already been described in over 120 original publications.

BEHAVIORAL CHANGES IN R6 MICE

The R6/1 and R6/2 mice display an array of behavioral and regulatory changes that develop gradually. Many behavioral changes that occur in R6/2 mice seem to appear also in R6/1 mice, to the extent that they have also been examined in R6/1 mice, which is by far the lesser studied model of the two. However, their onset is generally delayed by several weeks in R6/1 mice, and there is a slower progression of the severity of the symptoms. As will be described in more detail later, the level of environmental enrichment significantly influences the speed at which the behavioral phenotype evolves in R6/2 mice. Moreover, it appears that there are some differences in phenotype of R6 mice between colonies raised in different laboratories, possibly due to genetic drift, dietary factors, and/or housing conditions.[12] Therefore, it can be difficult to generalize regarding the age at which certain types of symptoms develop. Nevertheless, the majority of studies have been conducted in mice raised under standard laboratory conditions and therefore some comparisons of the ages of onset of different symptoms are valid. In R6/2 mice, the initial signs of motor symp-

toms commence around 3 weeks of age. The mice display locomotor hyperactivity at this stage.[14] Shortly thereafter, they exhibit the first signs of impairments of learning and memory in the Morris water maze test, which gradually becomes worse up to the age of 7 weeks when it is no longer possible to test them in this cognitive task due to severe motor deficits.[14–16] Whereas R6/2 mice are initially hyperactive, they gradually reduce their motor activity and become hypoactive around 8 weeks of age.[14,17] The mice begin to show an abnormal paw clasping response around the same time. When suspended by the tail, normal mice spread their four limbs, whereas R6 mice clasp their hind- and forelimbs tightly against their thorax and abdomen. The pathophysiology of this abnormal response is not fully understood. Nevertheless, the paw clasping test is often used in studies examining novel treatments, in part due to it being easy and fast to perform. Around the same age, R6/2 mice also begin to display other gradual changes in motor function such as stereotypical hindlimb grooming, changes in gait patterns and the emergence of some involuntary movements. As a result, their motor coordination progressively deteriorates, which can be detected as a reduction in the time they can stay on a rotating rod, the so-called Rotarod. Typically, R6/2 mice are severely impaired by 8–12 weeks of age.[14,17] In R6/1 mice a marked decline in Rotarod performance develops much later (around 13–20 weeks), and interestingly the degree of motor impairment seems to correlate with the numbers of striatal neurons exhibiting intranuclear inclusions of mutant huntingtin.[18]

The R6/2 mice in most colonies die at around 13–16 weeks of age, although some laboratories report that their R6/2 mice can live several weeks longer. There is less information on the expected life span of R6/1 mice, but they can definitely live for more than 1 year. Unfortunately, there is no comprehensive study describing the most common causes of death of R6/2 mice. Despite the lack of detailed information on causes of death, it is not uncommon for therapeutic studies to use prolongation of life as one of the main positive indicators of therapeutic efficacy in R6/2 mice. What could be the main causes of death in R6/2 mice? They clearly do not gain weight in a normal manner and there is muscle atrophy when they are around 8 weeks of age.[19] Part of the problem may be due to the fact that the mice experience difficulties eating regular lab chow, and this can be circumvented by giving them soft, palatable food in the bottom of the cages. Several studies have shown that a significant proportion of R6/2 mice gradually develop diabetes.[14,20] Initially they can produce insulin and only display impaired glucose tolerance. Gradually the insulin production fails and eventually they are hyperglycemic even after fasting.[21] R6/2 mice are prone to seizures that can be triggered by handling or unexpected noises.[1] It has been reported that the mice can die as a result of *status epilepticus*,[1] but it is not clear how common this is a cause of death and the underlying mechanism (either related to CNS pathology or electrolyte disturbances) is not understood.

BRAIN PATHOLOGY IN R6 MICE

Although there is a relative lack of understanding of the mechanisms behind the different behavioral features of the R6 mice, there is a wealth of information on how their brains undergo gradual pathological changes. Although most studies of novel therapies tend to focus on restoring brain size and inhibiting the formation of protein inclusions, in this section we will also briefly describe several of the other changes that occur. In an early characterization, it was reported that the brains of 12-week-old R6/2 mice weigh around 20% less than brains from wild-type controls.[22] Similarly, the volume of the striatum is reduced by 17% in 18-week-old R6/1 mice.[23] An intriguing feature of this marked reduction in brain volume is that until very recently there was very little cell death documented in the brains of R6 mice. In the cortex and striatum, a very small number of neurons undergo "dark cell degeneration,"[24,25] a morphological description of cell death that is believed to represent neither classical apoptosis nor necrosis. Recently, we reported that there is a progressive and dramatic loss of orexin-containing neurons in the lateral hypothalamus of R6/2 mice.[26] More recently, we also found that there is a reduction in the number of neurons expressing gonadotropin-releasing hormone in the hypothalamus and that this can cause the gonadal atrophy and infertility that develops in adult R6/2 mice (Papalexi, E., A. Persson, M. Bjorkqvist, A. Petersen, B. Woodman, G. Bates, F. Sundler, H. Mulder, P. Brundin, and N. Popovic, manuscript submitted). Although these hypothalamic changes are functionally important in relation to changes in reproductive, sleeping, and feeding behaviors, as is discussed below, the number of hypothalamic neurons that die is very small, and their disappearance cannot underlie the whole loss in brain volume in R6/2 mice. Most probably the significant reduction in brain volume is the result of atrophy of individual neurons and massive decrease in neuropil. In the R6/2 striatum, the cell bodies of medium-sized spiny neurons have been described to shrink by around 20% in surface area and the size of their dendritic fields is also reduced.[28] Similar reductions in neuronal size have been reported in the striatum and substantia nigra of R6/1 mice.[23,29]

A great deal of attention has focused on the development of intranuclear inclusions, containing the mutant truncated huntingtin, in neurons of R6 mice. Oddly enough, glial cells do not appear to develop these protein inclusions.[22] In R6/2 mice aggregates/inclusions first appear in the striatum and the cortex around 3–4 weeks of

age,[30,31] whereas they are not apparent in the R6/1 striatum until around 8 weeks of age.[18] The proportion of cells displaying inclusions varies between brain regions and increases gradually with age so that in some structures almost all neurons have inclusions at the terminal stage. For example, in the R6/2 striatum around 98% of the striatal projection neurons (calbindin positive) exhibit huntingtin inclusions at 15 weeks of age, whereas there are only few inclusions (1–2%) in certain neuronal types, such as somatostatin containing neurons.[31] Within the striatum, it appears that interneurons, in contrast to the efferent projection neurons, display fewer inclusions.[31,32] The inclusions are ubiquitinated, and the 20S subunit of the proteasome is recruited into the aggregates in R6/1 mice.[33]

Despite so few neurons actually dying in the brains of R6 mice, there is ample evidence that their brains do not function in a normal manner. For example, there are clear changes in gene expression in the striatum and cortex of R6/2 mice that have been documented as early as at 6 weeks and become more pronounced with age.[34] Numerous genes are altered and notably some striatal signaling genes induced by cAMP and retinoid are downregulated, whereas some genes associated with cell stress and inflammation (e.g., DNA repair enzymes) are upregulated. However, these changes do not appear to be specific for the brain regions that are classically affected in HD, but also occur in the cerebellum and in peripheral tissues such as muscle.[35] The concept that there is cell stress in the R6 striatum is supported by findings of increases in markers for oxidative damage to DNA,[36] transient increases in superoxide dismutase activity,[37] and reductions in mitochondrial function.[38] In addition, there is direct and indirect evidence for increased NOS activity, at least transiently, in the striatum of R6/1 and R6/2 mice.[38–41] In a cell culture study, we found that striatal neurons from R6/2 mice formed autophagic vacuoles in response to an oxidative insult more readily than control cells, suggesting a change in fundamental mechanisms related to the cell stress response in the R6/2 brain.[42]

There is also direct evidence for malfunction of the neuronal circuitry. In R6/2 mice, striatal neurons exhibit more depolarized resting potentials[43] and increased intracellular calcium levels[18] compared with wild-type controls, and there are changes in the firing patterns of corticostriatal fibers.[44] A number of studies have used the intracerebral microdialysis technique demonstrate changes in neurotransmitter release in the brains of R6 mice. Despite the fact that no changes in the capacity of the cells to synthesize and store neurotransmitters were reported, these studies pointed out possible alterations in the process of neurotransmitter release or reuptake.[29,45–47] For example, there is a reduction in the extracellular levels of dopamine in the striatum,[29] and an increase in extracellular striatal glutamate levels following stimulation,[45,47] at ages when the striatal tissue levels of these transmitters are normal. At later stages, the striatal tissue levels of dopamine are actually reduced in R6/2 mice.[48] Taken together, the complex changes in neurotransmission observed in R6 mice are difficult to explain by a single pathophysiological change. Most probably there are changes at multiple levels in neurons and glia. Thus, at specific stages of the disease progression in R6/1 and R6/2 mice, there is evidence for reduced capacity to synthesize neurotransmitters such as dopamine and serotonin;[49–51] changes in the levels of synaptic proteins;[52–56] alterations in a glial transport system that normally removes glutamate released from synapses.[46] Moreover, a strong body of evidence has highlighted changes in the postsynaptic elements involved in neurotransmission. In R6 mice, there is a progressive reduction in dopamine receptors and their downstream signaling partners,[57–59] and there is also evidence for changes in the different subtypes of glutamate receptors.[60]

An odd feature of the R6 mice is that they are partially resistant to neuronal damage following experimental lesions. For example, they display reduced susceptibility to neuronal death after intrastriatal injections of quinolinic acid;[23]NMDA[18] dopamine,[61] 6-hydroxydopamine[61] and the mitochondrial inhibitor malonate.[29,62] There is also reduced brain damage after a period of global cerebral ischemia[63] as well as systemic injections of kainic acid[64] and the mitochondrial toxin 3-nitropropionic acid.[65] Despite concerted efforts, it has not been possible to explain what underlies the neuroprotection at the cellular level. It appears unlikely that it is directly due to changes in e.g., NMDA receptor function, because striatal neurons in R6/2 slices allow entry of calcium when stimulated with an agonist such as quinolinic acid, but still do not die. The R6 mice exhibit functional changes in cortical inputs to the striatum that possibly also could contribute to the reduced sensitivity to neurotoxins.[43] The development of resistance to toxin-induced damage is age-dependent and more rapid in R6/2 than in R6/1 mice, and it appears to correlate with the appearance of nuclear inclusions.[18] Therefore, it may well be the result of a cellular mechanism that is central to the pathology of the R6 mice.

WHY HAVE THE R6 MICE BECOME POPULAR MODELS AND WHAT DISEASE MECHANISMS CAN BE STUDIED IN THEM?

The R6 mice were not only the first to be made available in the literature, but they were also rapidly placed into a commercial breeding facility where they could be accessed by most scientists. This undoubtedly promoted their use in studies of novel therapies. Moreover, the rapid disease progression in R6/2 mice, in terms of development of behavioral changes, brain pathology and

age of death, make them relatively easy and inexpensive to use. They clearly present the scientists with multiple outcome parameters to study, although the scientific community is still not certain about their relevance for the mechanisms underlying the disease in humans. There are several theories about what disease mechanisms are important in HD.[2,66–68] Briefly, it can be summarized that the majority of changes occurring in the brains of HD patients cause transcriptional dysregulation and/or alterations in protein folding and handling. These triggers have in turn led to cellular perturbations resulting in one or more of the following events: protein aggregate formation,[69] mitochondrial dysfunction and excitotoxicity,[70] synaptic dysfunction,[66] and cell degeneration or death through caspase activation (leading ultimately to apoptosis) and autophagy.[67] It can be argued that several, if not all, of these disease mechanisms are modeled in the R6 mice, albeit not perfectly. Because the R6 mice only express the N-terminal (*exon 1*) portion of huntingtin, a potentially crucial aspect of HD that is not modeled in these mice is the proteolytic cleavage of huntingtin. In the following sections, we describe the results from different experimental therapeutic trials in R6 mice. To facilitate an understanding of the usefulness of the R6 mice in this context, we have chosen to group the experimental treatment studies according to the fundamental disease mechanisms that they are believed to target (Table 1). Finally, we describe studies in which the treatment is restorative in nature, i.e., there is no attempt to inhibit the disease process directly, but instead the rationale is to replace lost functions by reparative strategies.

ACT ON PROTEIN MISFOLDING/ AGGREGATES TO PROTEASOME-CHAPERONE SYSTEMS

Chaperones, such as heat shock proteins (HSP), normally assist in folding proteins into appropriate conformations and are also capable of refolding already abnormally folded proteins. In cases when refolding fails, the misfolded protein can undergo ubiquitination. Polyubiquitination targets misfolded proteins to the proteasome where they are degraded.[69] It has been suggested that mutant huntingtin, with an expanded polyglutamine stretch that causes misfolding, can saturate the chaperone response. In addition, it has been speculated that mutant huntingtin can impair the ubiquitin-proteasome system by saturating it either by providing excessive substrate or by directly inhibiting the proteasome.[69,71–74] Recently, it was shown that the levels of Hdj1, HSP70,αSGT, and βSGT (small glutamine-rich tetratricopeptide repeat containing proteins) undergo a progressive decrease in the brains of R6/2 mice. By 14 weeks of age, they are reduced to around 40% of normal levels and this is considered to be due to recruitment of the chaperones into the huntingtin-positive inclusions.[75] Recently, we attempted to enhance chaperone activity and prevent disease progression, by crossing the R6/2 mice with mice overexpressing HSP70. The double-transgenic mice displayed a slight delay in the loss of body weight compared to regular R6/2 mice, but the HSP70 overexpression had no effect on the size of striatal neurons, the number of nuclear inclusions, and the loss of brain weight. It did not improve motor function.[76] Nevertheless, radicicol, a fungal antibiotic, and geldanamycn, a benzoquinone ansamycin, known to bind to HSP90 and to induce expression of HSP40 and HSP70 chaperones, have both been found to increase HSP response in culture models of HD and inhibit huntingtin aggregation.[75,77] However, these drugs have not yet been tested in R6 mice.

DRUGS TARGETING AGGREGATES AND AGGREGATE FORMATION

As mentioned earlier, one hallmark of HD is the presence of protein aggregates and R6 transgenic mice develop intraneuronal inclusions throughout the brain.[18,22,30,31] Several small molecules have been tested to inhibit aggregates/aggregate formation. *In vitro* studies have shown that Congo red can reduce aggregation of mutant huntingtin.[78,79] Importantly, Congo red has been reported to improve motor function, to reduce weight loss and to increase life span in R6/2 transgenic mice.[80] The same study reported that Congo red treatment inhibited polyglutamine oligomerization, prevented ATP depletion and caspase activation, preserved normal cellular protein synthesis and degradation in cells expressing mutant huntingtin. An intriguing feature of this study is that it suggested that Congo red could disrupt preformed polyglutamine aggregates.[80] Thus, Congo red administration of the R6/2 mice was initiated when the mice were 63 days old and already had developed intranuclear inclusions. When the treatment was terminated 2 weeks later, there were no huntingtin inclusions present in their brain. These unique data could be taken to suggest that the inclusions in R6/2 mice are really dynamic structures that can be efficiently degraded by the cells if there are no more huntingtin oligomers added. This concept of dynamicity of inclusions is supported by findings in a conditional transgenic model of HD where the transgene can be turned off by administration of tetracycline. When the expression of mutant huntingtin was turned on, neuronal inclusions formed in the cortex and the striatum. However, when expression of mutant huntingtin was turned off in mice that had already developed inclusions, the protein aggregates disappeared. This strongly suggests that continuous production of mutant huntingtin is required to maintain inclusion and that otherwise they are subjected to proteolytic breakdown.[81] Regarding the remarkable results obtained with Congo Red in R6/

TABLE 1. *Summary of Therapeutic Strategies that Have Been Tested in R6 Mice and their Effectiveness*

	Drug Delivery				Effectiveness	
Drug Name	Starting Age (Weeks)	Dose	Duration	Route of Administration	Increase in Survival (%)	Body Weight
Treatments targeting aggregates and aggregate formation						
Congo Red	9	6 μg/day in PBS and 0.2% DMSO	0.25 μl/h, 28 days	i.c.v., Osmotic pumps	16.4	Increased
Congo Red	9	1 mg/30g in PBS and 0.2% DMSO	Every 48 h	i.p.	16.4	Increased
Riluzole	3	10 mg/kg	Up to 16 weeks	Orally	10.2	17.2% increase
Trehalose	3	2%	Up to the end of the experiment (11–15 weeks)	Orally in drinking water	11.3	Decreased
Treatments targeting gene transcription						
SAHA	4	0.067% in 1.8% HOP-b-CD water solution	Up to 13 weeks	Orally, in drinking water	n.e.	No effect
Sodium butyrate	3	0.4–1.2 g/kg/day, dissolved in PBS (100 μl)	Up to 17 weeks	i.p.	20.8 at 1.2 g/kg/day	Increased only at the late stage (>11 weeks)
Mithramycin	3	150 μg/kg/day in PBS (100μl)	Up to death	i.p.	29.1	Increased
Transglutaminase as a therapeutic target						
Cystamine	7	100 μl of 0.01 M cystamine	7 days	i.p.	12	17.9–41.4% Increase at 10–14 weeks
Cystamine	3	112–225 mg/kg (100 μl/20 g/day)	14 Weeks	i.p.	19.5 (112mg/kg), 17.0 (225 mg/kg)	15.4% increase with 112mg/kg, 13.6% increase with 225 mg/kg
Cystamine	Prenatal	5 ml/day in 900 mg/liter drinking water (225 mg/kg)	3 Weeks	Orally	16.8%	12.7% Increase
Protease inhibitors						
Minocycline	6	5 mg/kg/day in 0.5 ml saline	Up to 13 weeks	i.p.	14	No effect
Tetracycline	6	5 mg/kg/day in 0.5 ml saline	Up to 13 weeks	i.p.	No effect	No effect
Minocycline	4	1–10 mg/ml, in drinking water with 5% sucrose	5–7 Days	Orally	n.e.	No effect
Inhibitors of apoptosis as a targeting strategy						
z-VAD-fmk	7	100 μg/20 g body weight	4 Weeks	i.c.v., Osmotic pumps	12.2–25	n.e.
YVAD-fmk	7	50 μg/20 g body weight	4 Weeks	i.c.v., Osmotic pumps	No effect	n.e.
DEVD-fmk	7	50 μg/20 g body weight	4 Weeks	i.c.v., Osmotic pumps	No effect	n.e.
YVAD-fmk and DEVD-fmk	7	50 μg/20 g body weight	4 Weeks	i.c.v., Osmotic pumps	17.3%	n.e.
Coenzyme Q10	3	0.2% in the diet (400 mg/kg/day)	Up to 13 weeks	Orally	14.5	12.7%
Remacemide	3	0.007% in the diet (14 mg/kg/day)	Up to 13 weeks	Orally	15.5%	10.1
Combined coenzyme Q10 and remacemide	3		Up to 13 weeks	Orally	32%	20.3
Targeting excitotoxicity						
LY379268	3.5	1.2 mg/kg Dissolved in water	Up to 10 weeks	Orally	10.5	No effect
MPEP	3.5	100 mg/kg Dissolved in water	Up to 10 weeks	Orally	15.5	No effect
Treatments targeting energy metabolism and diet						
Creatine	3	1,2, or 3% Diet supplementation	Up to 13 weeks	Orally	9.4 in 1%, 17.4 in 2%, 4.4 in 3% creatine	7.8, 10.3, and 6.5% Increase in 1, 2 and 3% creatine, respectively
Creatine	6, 8, or 10	2% Diet supplementation (4 mg/kg)	Until death	Orally	14.4% and 9.7% in 6- and 8-week groups, respectively	18.7% Increase for the 6 weeks starting group
Unsaturated fatty acids			Throughout life	Orally	n.e.	Increased
Anti-inflammatory agents						
Acetylsalicylate	From weaning	200 mg/kg/day	Until death	Orally	Decreased	No effect
Rofecoxib	From weaning	15 mg/kg/day	Until death	Orally	No effect	No effect
BN82451	4	30 mg/kg/day	Until death	Orally	15.3	No effect
Other drugs						
TUDCA	6	500 mg/kg, Once every 3 days	Until death	s.c.	n.e.	n.e.
Lithium	5 or 10	10.4–16 mg/kg/day	up to 15 weeks	s.c.	No effects	Decreased in 5-weeks group; increased in 10-weeks group
Ascorbate	6	300 mg/kg, 4 Days per week	3 weeks	i.p.	n.e.	n.e.
GDNF	4–5	Lentiviral vector		n.e.	n.e.	No effect
AsialoEPO	5	80 μg/kg	7	i.p.	n.e.	No effect
Double-transgenic models						
Dominant-negative mutant of caspase-1	Conception				20%	Delayed loss of body weight
HSP70	Conception				No effect	Delayed loss of body weight
Bcl-2	Conception				10.3%	n.e.
Repair strategies						
Striatal transplant	10	E13–14 LGE striatal anlage	6 Weeks survival post operation		n.e.	No effect
Anterior cingulate cortical transplant	Birth R6/1				n.e.	n.e.

(*Table continues*)

TABLE 1. *(Continued)*

Effectiveness						
Brain Weight	Number of Aggregates	Blood Glucose Level	Locomotor Activity (Open Field, etc.)	Motor Coordination (Rotarod, etc.)	Brain Atrophy	Refs.
n.e.	Decreased	55.3% Decreased	Improvement in "ink" test	Improved	n.e.	80
n.e.	Decreased	55.3% Decreased	Improvement in "ink" test	Improved	n.e.	80
n.e.	Less ubiquitinated	No effect	29% Improvement, between 4 and 6 weeks	No effect	n.e.	83
4.2% Increase	Decreased	No effect	Improvement in foot printing test	Improved	Decreased atrophy	84
n.e.	No effects in hippocampal slice culture	n.e.	No effects in grip strength test	Improved	Improved	89
Increased with 1.2 g/kg/day	No effect	n.e.	n.e.	Improved	Improved by one fold	90
Increased	No effect	n.e.	n.m.	Improved by 42.6%	Improved	91
n.e.	No effect	No difference in urine	29% Delay of tail clasping	Improvement in hind-paw print pattern	n.e.	95
Increase	68% Decrease in the striatum and 47% decrease in the neocortex.	n.e.	n.e.	Improved by 27%	Improved	96
n.e.	n.e.	n.e.	n.e.	Improved	n.e.	96
n.e.	No effect	No effect	n.e.	Improved	No effect	104
n.e.	No effect	No effect	n.e.	No effect	No effect	104
n.m.	No effect	Decreased	No effect in grip strength	No effect	n.e.	106
n.e.	n.e.	n.e.	n.e.	Improved	n.e.	104 113
n.e.	n.e.	n.e.	n.e.	No effect	n.e.	104
n.e.	n.e.	n.e.	n.e.	No effect	n.e.	104 113
n.e.	n.e.	n.e.	n.e.	Improved	n.e.	104 105
Delayed loss by 16.1%	8.2% Decrease at week 9, 15.7% at week 13	n.e.	n.e.	Improved by 44.5%	Delayed by 52.8%	119
Delayed loss by 16.9%	8.2% Decrease at week 9; 15.7% at week 13	n.e.	n.e.	Improved by 54.7%	Delayed by 52.9%	119
Delayed loss by 17.5%	32% Decrease at week 9; 36% at week 13	n.e.	n.e.	Improved by 62.2%	Delayed by 87.8%	119
n.e.	No effect in number Increase in size in the cortex	No effect	Improvement in early hyperactivity at 4–6 weeks	No effect	n.e.	125
n.e.	No effect in number; Larger inclusions in the cortex	No effect	Improved at early hyperactivity at 4–6 weeks	Improved	n.e.	125
17% Increase in 2% creatine at day 90	35–60% Decrease in striatum in 4–13 weeks in 2% creatine	Decreased in 2% creatine	n.e.	25, 33, and 6.5% Improvement in 1, 2 and 3% creatine, respectively	Delayed	126
17.4% Increase for the 6 weeks group	39% Decrease in the striatum in the 6 weeks starting group	n.e.	n.e.	23% and 19% Improvement in 6- and 8-week groups, respectively	Delayed in the 6-week starting group	128
n.e.	n.e.	n.e.	Increase in rearing, decrease in grooming	Decrease in paw clasping	n.e.	131
n.e.	n.e.	n.e.	n.e.	No effect	No effect	135
n.e.	n.e.	n.e.	n.e.	No effect	No effect	135
n.e.	Decreased	n.e.	n.e.	Improved	Reduced	136
n.e.	Decreased	n.e.	Improved	Improved	Reduced	139
n.e.	n.e.	n.e.	n.e.	No effect in 5-week group; improved in 10-week group	n.e.	141
n.e.	n.e.	n.e.	Decrease in grooming	Increase in the cognitive performace test	n.e.	143
n.e.	No effect	n.e.	No effect	No effect	No effect	145
n.e.	No effect	n.e.	No effect	No effect	No effect	161
n.e.	Delayed appearance of inclusions	n.e.	n.e.	Improved	n.e.	113
n.e.	No effect	n.e.	No effect on paw clasping	n.e.	No effect	76
n.e.	n.e.	n.e.	n.e.	Improvement at 6 and 11 weeks	n.e.	116
n.e.	n.e.	n.e.	Minimal effects	n.e.	n.e.	154
n.e.	n.e.	n.e.	No effect	Delayed rear-paw clasping symptoms	No effect	153

n.e. = not evaluated; i.c.v. = intracerebroventricular; i.p. = intraperitoneal; s.c. = subcutaneous; SAHA = suberoylanilide hydroxamic acid; MPEP = 2-methyl-6-(phenylethynyl)-pyridine; zVAD-fmk = Val-Ala-Asp-fluoromethyl ketone; YVAD-cmk = Tyr-Val-Ala-Asp-chloromethylketone; DEVD-fmk-Asp-Glu-Val-Asp-aldehyde-fmk; TUDCA = tauroursodeoxycholic acid; GDNF = glial derived neurotrophic factor; asialoEPO = asialoerythropoietin; HSP70 = heat shock protein 70; LGE = lateral ganglionic eminence; E13 = embryonic day 13.

mice,[80] there is debate concerning their interpretation. Surprisingly, the effects of systemic Congo red treatment were similar to those obtained when the drug was administered into the cerebral ventricles, despite prior claims by others that Congo red does not pass the blood brain barrier efficiently. An alternative interpretation of these data are that systemically administered Congo red did affect huntingtin aggregation in peripheral tissues, suggesting that targeting huntingtin aggregation in peripheral tissues could be important for the motor function and life span of R6/2 mice.

Benzothiazoles derivatives, including riluzole, which is a glutamate release inhibitor, have neuroprotective effects and inhibit aggregate formation *in vitro*.[82] Riluzole orally administered to 3-week-old R6/2 mice improved survival by 10%, and delayed weight loss by 17%. There was no clear difference in frequency and size of aggregates in cortical neurons between riluzole-treated and transgenic controls. In contrast, the number and the size of intranuclear aggregates were decreased in the striatum of riluzole-treated mice. Furthermore, during the hyperactive phase (4–6 weeks of age) of R6/2 mice, riluzole significantly attenuated the spontaneous locomotor activity. However, there was no significant improvement in motor coordination in the Rotarod test.[83]

A recent study demonstrated that various disaccharides could inhibit polyglutamine aggregate formation. The most effective disaccharide, trehalose, when administered orally via drinking water to 3-week-old R6/2 mice, substantially reduced aggregate formation in different brain regions and even in cells in the periphery, such as the liver. Animals treated with trehalose exhibited improved motor function, less brain atrophy, and prolonged life span by 11%.[84] Trehalose is normally present in cells and metabolized to glucose. The mechanisms underlying neuroprotective effects of trehalose are still not clear. It has been suggested that trehalose may bind directly to the expanded polyglutamine and inhibit aggregation. In addition, trehalose may stabilize the protein so it does not undergo proteolysis by caspases and thereby trehalose may prevent the translocation of truncated huntingtin to the nucleus. However, this particular mechanism is less relevant in the R6/2 model where only *exon* 1 of huntingtin is expressed.

TREATMENTS TARGETING GENE TRANSCRIPTION

Huntingtin is normally distributed in the cytoplasm as well as in the nucleus.[85,86] As mentioned earlier, mutant huntingtin can be proteolytically cleaved and is targeted into the nucleus to some degree. In the R6 mouse models of HD, the intranuclear localization of the mutant protein is an early and prominent event and therefore interference with gene transcription could be a particularly prominent feature in these mouse models. Indeed, they display widespread and progressive transcriptional changes in both brain and peripheral tissues, as evidenced by microarray studies.[34] Mutant huntingtin is thought to specifically interact with various transcriptional activators and coactivators, including the cAMP response element binding protein and the specificity protein 1[68] and disruption of transcriptional pathways could occur through interactions between mutant huntingtin and those nuclear proteins.[87] The acetylation and deacetylation of histones in nucleosomes are also important in regulation of gene expression, evidence has suggested that these processes may be altered in HD. The levels of acetylated histones H3 and H4 are decreased in animal models of HD and these changes have also been suggested to be central to polyglutamine protein pathology. Inhibitors of histone deacetylase (HDAC) can reverse the reduction in acetylated histones in a *Drosophila* model of HD and thereby reduce cell death.[88] HDAC inhibitors have also been tested in trials in R6/2 mice. Suberoylanilide hydroxamic acid (SAHA), a selective inhibitor of histone deacetylase, increased histone acetylation and ameliorated motor deficits when given systemically to R6/2 mice in a special formula designed to cross the blood brain barrier.[89] However, this study did not show that histone acetylation was reduced in the brains of the R6/2 mice under baseline conditions, so the primary target of the SAHA treatment may not have been brain cells in this particular model. Also, the effects of the SAHA treatment were not dramatic, and only evident as a minor improvement, albeit significant with the statistical tests employed, in Rotarod performance and muscle grip strength. In another study, sodium butyrate, another HDAC inhibitor, improved survival of R6/2 mice and mitigated body weight loss and decreased atrophy of the striatum in a dose-dependent manner.[90]

Mithramycin, a clinically approved guanosine-cytosine-rich DNA binding antitumor antibiotic used for the treatment of hypercalcemia and several types of cancers. Systemic treatment of R6/2 transgenic mice with mithramycin extended survival of the mice by almost 30% with improved motor performance in the Rotarod test and indications of reduced neuropathological changes. At 3 weeks of age and following about 9 weeks of treatment, the reduction of brain weight loss significantly mitigated from 21% in transgenic controls to around 3% in the mithramycin-treated R6/2 mice. In addition, mithramycin could prevent brain atrophy, neuronal size was clearly larger in treated mice than in the untreated transgenic controls (over 100% increase in cell body surface area).[91] The mechanism of action underlying the effects of mithramycin include increased methylation of lysine 9 in histone H3, a well-established mechanism of gene silencing. This prevented the increase in H3 hypermethylation observed in R6/2 mice, suggesting that the en-

hanced survival and neuroprotection might be attributable to the alleviation of repressed gene expression vital to neuronal function and survival.[91]

TRANSGLUTAMINASE AS A THERAPEUTIC TARGET

Transglutaminase has been shown to selectively polymerize huntingtin[92] and promote the aggregation of huntingtin into nonamyloidogenic polymers.[93] Therefore, it has been suggested that transglutaminase plays a central role in aggregate formation in HD. The level of transglutaminase has been reported to be increased in the post-mortem human brains and transgenic mouse models of HD.[93–95] Therefore, transglutaminase is an interesting target for possible therapeutic intervention. In a recent study, R6/2 mice received systemic injections of cystamine, a transglutaminase inhibitor, for 7 days. This treatment reduced the transglutaminase level by 36%. Inhibition of transglutaminase activity was observed as early as 10 min after a single injection. The mice receiving cystamine exhibited less tremor, decreased abnormal movements and delayed onset of paw clasping by around 20 days. At the same time, body weight increased by 18–41% between 10 and 14 weeks of age. In addition, cystamine treatment increased survival by 12%, but without affecting the frequency and distribution of nuclear inclusions in the brain.[95] In another study, systemic cystamine treatment of R6/2 mice extended life span (17–20%), improved motor function (by 27% as assessed by the Rotarod test), reduced aggregate formation (by 68% in striatum and 47% in neocortex) and attenuated brain atrophy.[96] The mechanism of action of cystamine is thought to involve inhibition of transglutaminase mediated cross-linking of mutant huntingtin and thereby prevention of aggregate formation. In addition, a recent study has shown that cystamine can also increase intracellular levels of antioxidant L-cysteine in the brain.[97] Because oxidative stress plays an important role in HD pathogenesis, increased levels of L-cysteine after administration of cystamine could be neuroprotective in HD.[97] Thus, cystamine could play a dual role by inhibiting transglutaminase and acting as an antioxidant.

PROTEASE INHIBITORS AS THERAPEUTIC AGENTS IN HD

Various proteases, such as caspases,[98,99] calpains,[100] and aspartyl proteases[101] can cleave huntingtin, and thereby promote aggregate formation and increase cell toxicity. Elevated activities of those proteases have been observed in the brains of patients and transgenic mouse models of HD.[98,100–102] Mutation of calpain cleavage sites renders the expanded polyglutamine huntingtin less susceptible to proteolysis and aggregation resulting in decreased cellular toxicity.[100] These findings support the idea that proteases play an important role in huntingtin proteolysis and toxicity, and open new windows for possible therapeutic strategies. In theory, protease inhibitors can reduce the accumulation of N-terminal fragments of mutant huntingtin and therefore, prevent or delay disease progression. As mentioned earlier, in relation to the R6 mice, the issue of huntingtin cleavage is less relevant, because these mice only express *exon* 1 of the gene. Thus, they only express a short N-terminal fragment of the protein that does not include, e.g., the caspase cleavage site that has been identified around amino acid 552.[103] Consequently, treatments with drugs that inhibit proteases cannot act primarily through inhibiting proteolytic cleavage of huntingtin itself in the R6 mice. Instead, in the R6 models protease inhibitors would target, e.g., caspase activation that could occur as a downstream consequence of the toxic effects of the N-terminal fragment of mutant huntingtin, which are not yet fully understood. A modified tetracycline antibiotic, minocycline, a caspase inhibitor with anti-inflammatory properties, was shown to inhibit huntingtin aggregate formation and prolong survival of R6/2 mice.[104,105] Minocycline was initially shown to inhibit activity of nitric oxide synthetase and up-regulate caspase-1 and caspase-3 mRNA, when administered intraperitoneally in R6/2 mice from the age of 6 weeks. Although no effect on inclusion formation was observed, disease progression was delayed, survival time was extended by 14%, and motor function was improved in the Rotarod test.[104] Further analysis of the antiapoptotic properties of minocycline have suggested that it involves inhibition of caspases and mitochondrial cytochrome C and Smac/Diablo release, as well as other caspase-independent mechanisms that are still not completely understood.[105] In contrast to the positive effects of minocycline treatment presented above, a more recent paper reported a lack of neurological improvement and even some toxicity when minocycline or doxycycline were administered to R6/2 mice via the drinking water.[106] In this study, minocycline had no effect on aggregate formation. However, when applied to hippocampal slice cultures derived from R6/2 brains, minocycline was effective at reducing aggregate formation.[106] Possibly, the lack of effect in the mice *in vivo* was related to the route of administration which was oral in the follow-up study[106] and differed from the intraperitoneal administration used in the initial one[104] Despite the caution expressed in these latter studies on the effects of minocycline in R6/2 mice, minocycline has been recently used in the clinical HD trials.[107–110] Among these trials, the study on 11 HD patients by Bonelli and colleagues has the longest follow up of 2 years[108] In a preliminary report, the authors suggest that minocycline stabilized general motor, psychological and psychiatric functions in treated HD patients.

INHIBITORS OF APOPTOSIS AS A TARGETING STRATEGY

Although the mechanisms of neuronal injury and death are still unknown in HD, they are thought to include glutamate-mediated excitotoxicity and mitochondrial dysfunction. Both may lead to an increased production of free radicals. Cytochrome C release into the cytoplasm[111] and activation of caspases 1, 3, 8, and 9[111–113] were observed in HD patients and animals models, indicating that apoptosis indeed plays an important role in HD pathogenesis. In addition, as mentioned above, the effects of minocycline may be related to inhibition of both caspase-independent and -dependent mitochondrial cell death pathways.[105,114] The caspase inhibitor, z-VAD-fmk has been used in R6/2 transgenic mice and shown to improve survival and motor function by 12–25% when delivered into the lateral ventricle.[104,113] Additive effects of YVAD-fmk and DEVD-fmk were also observed. When administered intraventricularly, these combination of caspase inhibitors led to a prolonged survival (by 17%) of R6/2 mice.[104,105] Expression of a dominant-negative caspase 1 mutant in R6/2 mice has also been reported to extend survival and delay the appearance of neuronal inclusion, neurotransmitter receptor alterations, and onset of symptoms, indicating that caspase 1 activation is important for the disease development in R6/2 mice.[113] However, it should be noted that caspase 1 is not directly involved in the apoptotic pathways, but considered to act as a proinflammatory player.[115] In addition, there is no clear evidence of apoptosis in the brains of R6/2 mice. Taken together, the effects of dominant negative caspase 1 in R6/2 mice may not be related to apoptosis. Using the similar strategy, this group also evaluated the role of Bcl-2 family members in the HD pathogenesis. After crossing R6/2 mice with transgenic mice selectively overexpressing Bcl-2 in neurons under the control of neuron-specific enolase promoter, the double-transgenic mice showed significant delay in onset of motor deficits and prolonged life span by 10.3%.[116] Furthermore, in the experiments with administration of z-VAD-fmk and YVAD-fmk, these enzyme inhibitors may also have led to a general reduction in the activity of cellular proteases as they are not caspase-specific at higher concentrations. Therefore, they may have inhibited other signaling pathways than those directly related to caspase-mediated cell death.

Defects in mitochondrial function may contribute to the pathogenesis of HD. Studies with purified mitochondria demonstrated that mutant huntingtin can associate with the outer mitochondrial membrane and directly induce the opening of the mitochondrial permeability transition pore with simultaneous release of cytochrome C.[117] In addition, deficits of mitochondrial complexes I, II, and IV have been observed in the brains of HD patients and animal models. Several drugs that can enhance mitochondrial functions have been tried in clinical trails and animal studies. Coenzyme Q10, a cofactor of the electron transport chain and an antioxidant, significantly decreased cortical lactate concentration in HD patients[118] and protected against striatal lesions induced by the mitochondrial toxins, malonate, and 3-nitropropionic acid. Oral administration of either coenzyme Q10 or remacemide, an NMDA antagonist, to transgenic animals including R6/2 mice can prolong survival by 10–12%, significantly delay motor deficits, reduce weight loss, and aggregate formation.[119] Furthermore, a combined treatment of coenzyme Q10 with remacemide was shown to have additive effects, promoting the recovery of motor dysfunction, attenuating ventricular enlargement, and increasing the survival of R6/2 mice.[119,120] Unfortunately, a large clinical trial with chronic treatment of coenzyme Q10 and remacemide hydrochloride showed no significant slowing of functional decline of early stage HD patients with either drugs given alone or in combination. This indicates that studies in R6/2 mice and other animal models of HD may not accurately predict the outcome of clinical trials.[121]

TARGETING EXCITOTOXICITY WITH DRUG THERAPY IN R6 MICE

The overactivation of NMDA glutamate receptors has long been suggested to cause overexcitation of striatal neurons, ultimately leading to their death in HD.[122] If this is really the case, it is not clear that it is reflected in the phenotype of R6 mice. Decreases in total levels of striatal glutamate and its receptors have been determined in R6/2 mice.[57,123] In a microdialysis study, however, we have observed increased extracellular levels of glutamate in the striatum following potassium-induced depolarization in 16-week-old R6/1 mice.[45] Those observations are in agreement with other studies indicating that the glial glutamate transporter-1 are reduced in R6 mice, thereby effectively reducing the uptake of synaptically released glutamate.[46] Moreover, electrophysiological studies on the corticostriatal pathway in brain slices prepared from R6/2 mice[44,124] indicate that there are complex changes in glutamatergic transmission. Taken together, it appears that changes in glutamate could be important in the development of the neurological phenotype in R6 mice, but there is no clear evidence for ongoing excitotoxicity. Interestingly, oral administration of the glutamate antagonist, riluzole, has been reported to increase the survival of mice by 10% and to decrease body weight loss by 17%. However, there were no remarkable effects on motor coordination except for a 29% reduction in early motor hyperactivity.[83] In a follow-up study, oral administration of either MPEP, postsynaptic metabotropic glutamate receptor 5 antagonist, or inhibition of glutamate

release by LY379268, a mGluR2 agonist, significantly increased the survival of R6/2 transgenic mice for 2 weeks (10% of life span).[125] These treatments reduced motor hyperactivities at 4–6 weeks of age and there was an approximate 1- to 2-week delay in the decline in Rotarod performance following both treatment paradigms. Both treatments also resulted in significant increases in the diameter of EM48-positive huntingtin inclusions in the cerebral cortex, with similar trends in the striatum. However, the interpretation of this increase in inclusion size is not clear. In summary, there is evidence that glutamatergic neurotransmission is significantly affected in R6 mice, and that treatments with different glutamate antagonists can affect development of the phenotype, albeit only to a minor degree.

THERAPEUTIC TREATMENTS TARGETING ENERGY METABOLISM AND DIET

There is substantial evidence for bioenergetic defects in HD. Creatine administration increases brain phosphoreatine levels, which stabilizes the mitochondrial permeability transition, prevents ATP depletion and stimulates protein synthesis or reduces protein degradation. In R6/2 transgenic mice, addition of 2% creatine in diet substantially increased survival, delayed development of motor deficits, reduced weight loss, attenuated brain atrophy, and inhibited aggregate formation.[78,126–128] However, in a pilot clinical trial, after 1 year of creatine intake, there was no clear improvement of functional and cognitive status in HD patients with grades I–III.[129]

Alterations in lipid metabolism have been associated with neurodegenerative processes affecting the striatum, and similar changes have also been suggested to occur in R6 mice. This idea was supported by a report in which striatal lipid peroxidation correlated with the progression of neurological phenotypes of R6/1 mice.[130] Essential fatty acids, such as linoleic acids of N-3 and N-6 series, have been shown to exhibit antidyskinetic properties. Therefore, the effects of supplementation of the diet with essential fatty acids have been tested in R6/1 mice, with the special diet given every second day from conception till adulthood. The diet was composed of 48% linoleic acid, 6% γ-linolenic acid, 5% α-lipoic acid, and 3% d-α-tocopherol acetate. This treatment protected against motor deficits reducing the incidence of "feet clasping" by 50% and completely preventing the reduction of stride length as the R6/1 mice grew older. In addition, the essential fatty acid treatment also extended the survival of treated mice.[131] The mechanism underlying the protective effects of highly unsaturated fatty acids is not well understood. It is known that the lipid constituents of cell membranes play important roles in regulation of neuronal signaling. Although it has been speculated that essential fatty acids may arrest huntingtin aggregation, inhibit histone deacetylase and/or activate the ubiquitin-proteosomal system, these hypotheses need further validation.[132] Significant palliative effect of administration of mixtures of unsaturated fatty acid has been suggested to take place in controversial, small clinical trials on HD patients. The patients have been reported to exhibit improvements in motor and cognitive performance after treatment.[133,134]

ANTI-INFLAMMATORY AGENTS TESTED IN R6 MICE

Inflammatory mechanisms have been implicated in the pathogenesis of HD. Gene array analysis showed increased expressions of genes associated with inflammation in R6/2 mice in 6–12 weeks of age.[34] As mentioned earlier, R6/2 mice lived longer when they were crossed with transgenic mice with a dominant-negative inhibitor of the proinflammatory cytokine caspase 1 (also known as interleukin 1β-converting enzyme).[113] Minocycline, which is known to inhibit microglia activation, had also neuroprotective effects in R6/2 mice. Recently, acetylsalicylate or rofecoxib, anti-inflammatory drugs affecting cycloxygenase 1 and 2, respectively, have been orally administered in transgenic mouse HD models. After treatments in the R6/2 mice, both drugs failed to show effects on survival, weight loss, and behavioral abnormalities.[135] However, another anti-inflammatory and antioxidant compound, BN82451, significantly, but transiently, improved Rotarod performance in R6/2 mice when given orally starting before the onset of symptoms. Onset of symptoms was postponed and survival extended for around 2 weeks.[136] Brain atrophy and ventricular enlargement were significantly smaller at treated group, and accompanied by reductions in neuronal intranuclear formations and neuronal atrophy.

The effects of other drugs in R6 mice

There are several drugs that cannot be classified according to a specific type of mechanism of action. In this section, we summarize the effects of such drugs that have been already tested in R6/2 mice.

In vitro experiments have pointed out that hydrophilic bile acids can exhibit neuroprotective effects. Tauroursodeoxycholic acid (TUDCA), for example, reduces mitochondrial membrane perturbation, cytochrome C release and caspase activation.[137,138] Subcutaneous administration of TUDCA in R6/2 mice after the onset of symptoms reduced striatal atrophy as well as the frequency and average size of huntingtin inclusions. The authors also reported that there was a reduction in the number of TUNEL-positive, apoptotic cells in the striatum. This result is difficult to interpret because the generally accepted view is that there are virtually no apoptotic cells in the striatum of R6 mice. TUDCA treatment

was also suggested to improve motor abilities, causing increased locomotion in the open field test and improving the performance in the Rotarod test.[139]

Neuroprotective properties of lithium have also been examined in R6/2 mice. Lithium is known to inhibit inositol monophosphatase, thereby reducing phosphatidylinositol synthesis, and to increase expression of the antiapoptotic factor Bcl-2. Lithium has also been suggested to inhibit polyglutamine toxicity via inhibition of glycogen synthetase (GSK-3) and increase in glutamate uptake, therefore preventing excitotoxicity.[140] Lithium was given both to presymptomatic (from about 5 weeks of age), and postsymptomatic (2 days after the appearance of hindlimb grooming) mice. This treatment significantly improved Rotarod performance, but not weight loss and survival, of the mice in the symptomatic group. The effects on neuropathology were not evaluated. In presymptomatic mice, there was a significant decrease in body weight after 3 weeks of treatment and a lack of motor improvements. Lithium, therefore, had rather different effects in R6/2 mice depending on when the drug is given.[141]

Ascorbate is an antioxidant vitamin and the levels of extracellular ascorbate in the striatum normally appear to be related to behavioral responses. Extracellular ascorbate is reduced in the striatum of R6/2 mice.[142] When animals were treated with intraperitoneal injections of ascorbate, 4 days per week, from 6–10 weeks of age, there were significant reductions in stereotypic grooming and an increase in cognitive performance was detected. Unfortunately, the authors did not evaluate whether ascorbate administration had any effects on life span, aggregate formation, and neuropathology of R6/2 mice.[143]

TROPHIC FACTOR DELIVERY AS AN OPTION

Various neurotrophic factors have been shown to be neuroprotective in the excitotoxic models of HD, typically when administered before injection of the toxin.[144] We recently showed that long-term lentiviral delivery of glial cell line-derived neurotrophic factor (GDNF) into the striatum of R6/2 mice, starting around the onset of motor symptoms, did not significantly affect Rotarod performances, open field behavior or neuropathologic changes.[145] There is growing evidence suggesting that BDNF synthesis and axonal transport are impaired in HD.[146–149] Wild-type huntingtin up-regulates transcription of BDNF. This beneficial activity of huntingtin is lost when the protein is mutated, resulting in decreased production of BDNF in cortical neurons.[146] A more recent study has demonstrated that neuron restrictive silencer element (NRSE) is the target of wild-type huntingtin activity. Under normal conditions, huntingtin promotes the cytoplasmic sequestering of repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF) in the cytoplasm and prevents the suppression of, e.g., the BDNF promoter. In HD, it is suggested that there is not only a toxic gain-of-function of the mutant protein but also a partial loss-of-function of wild-type huntingtin. In agreement with this concept, a reduced expression of NRSE-controlled neuronal genes, including BDNF, was observed in cellular and animal models of HD, as well as HD patients.[150] Reduction of BDNF protein in the caudate-putamen of HD patients ranges from 53–82%.[146,151] If R6/1 mice are crossed with +/− BDNF mice, the offspring that have reduced BDNF levels exhibit more rapid disease progression than regular R6/1 mice.[148] Therefore, increasing BDNF levels by either enhanced endogenous production or exogenous delivery might be viable therapeutic approaches in HD. This hypothesis is supported by a finding that striatal neurons in R6 mice are still responsive to BDNF. Thus, BDNF application to medium-sized spiny neurons in striatal slices prepared from R6/1 and R6/2 mice, significantly reduced GABAergic synaptic currents.[152] Recently, Canals and co-workers[148] have evaluated the effect of BDNF administration on the neurological phenotype of R6/1 mice. Using osmotic mini-pumps, BDNF was infused unilaterally into the striatum of R6/1 mice for 1 week, starting at 20 weeks of age. The treatment increased the immunoreactivity in enkephalinergic neurons by 60% above transgenic controls, without affecting substance P-positive neurons. However, the possible effects of BDNF on striatal atrophy and inclusion formation was not examined in this study. Clearly, it would be interesting to study the effects of long-term BDNF overexpression in R6 mice.

DOES CELL THERAPY WORK IN THE R6 MICE?

Implantation of neural tissue from wild-type donors into the brain of transgenic R6 mice has also been evaluated as a therapeutic approach. Neonatal anterior cingulate cortex from wild-type donors has been grafted homotopically to the cortex of neonatal R6/1 mice.[153] This approach was based on the idea that the cingulate cortex is an important area of the HD pathology and that the transgenic cortex could exert an excitotoxic influence on the striatum. However, the grafts had a minor ameliorative effect on paw clasping and did not affect all the other motor behaviors studied.[153]

In another set of experiments, cell suspensions derived from embryonic ganglionic eminence, which contains the cells that normally form the striatum, were grafted into the striatum of 10-week-old R6/2 mice.[154] These mice were already relatively advanced of the disease, and although the grafts survived well, they only had minor

beneficial effects in the multiple behavioral parameters that were examined. Because R6 mice have so little striatal and cortical cell loss, one can therefore question whether those mice are good model to test cell replacement strategies for HD.

ENRICHED ENVIRONMENT

Enriched environment and physical activity have been shown to negatively correlate with incidence and progression of several neurological diseases. Mimicking conditions that are closer to the environment existing in the natural wild for rodents has been tested. For example, R6 mice have been housed in cages containing several mice, cardboard tubes and other "toys," as well as food pellets on the cage floor. This type of enriched environment has repeatedly been shown to improve Rotarod performance and paw clasping, and slow the disease progression.[59,155,156] Importantly, these beneficial effects were neither due to an increased muscular strength nor to weight gain. Interestingly, the level of enrichment did not influence the observed improvement, because minimal enrichment, consisting only in food supply on the cage floor, and a maximal enrichment including additional components of the dominance hierarchy organization of their social life, led to similar beneficial results.[155–159] Environmental enrichment is known to enhance synaptic plasticity, promote hippocampal neurogenesis and increase learning performance, which might contribute to the positive effects in R6 mice. In recent follow-up studies, it has been found that environmental stimulation has profound effects on several neuropathological markers in R6 mice. Thus, the enriched environment prevented the loss of striatal volume, increased striatal and hippocampal BDNF content, mitigated the cortical deficit in DARPP-32[159] and delayed the loss of cannabinoid CB1 receptors in the basal ganglia.[157] However, there appear to be no changes in aggregate accumulation[59,156] and striatal dopamine D1 and D2 receptors[157] after enriched environment in R6 mice.

NEUROGENESIS AS A TARGET

Over recent years, it has become clear that there is neurogenesis in certain regions of the adult brain. The two major areas that exhibit neurogenesis are the dentate gyrus of the hippocampus and the subventricular zone adjacent to the lateral ventricles, with the latter region providing neural precursors that migrate to the olfactory bulb. Increased neurogenesis was recently reported to occur in the subventricular zone adjacent to the caudate nucleus in HD patients. The increase in cell proliferation correlated with the number of CAG repeats and severity of the disease.[160] In contrast, reduced hippocampal cell genesis has been reported both in R6/1 and R6/2 mice,[161,162] with no evidence of alterations in cell proliferation in the subventricular zone. In a more recent and detailed study in R6/2 mice, we have established that there is indeed a reduction in the number of newborn hippocampal neurons and that this deficit appears already at a presymptomatic stage (Gil, J. M., P. Mohapel, I. M. Araujo, N. Popovic, J. Y. Li, P. Brundin, and A. Petersen, manuscript submitted). We also tested the effects of asialoerythropoetin, a variant of the cytokine erythropoetin that is known to be neuroprotective and promote cell proliferation.[163] Given systemically from 5–12 weeks of age, asialoerythropoetin had no effects on the reduced hippocampal neurogenesis observed in R6/2 mice. The treatment had also no effects on paw clasping and Rotarod performance, weight loss, striatal atrophy, and striatal neuronal atrophy, nor in the number of striatal intranuclear inclusions (Gil, J. M., P. Mohapel, I. M. Araujo, N. Popovic, J. Y. Li, P. Brundin, and A. Petersen, manuscript submitted).[161]

PROS AND CONS OF ANIMAL STUDIES AND RELEVANCE TO HUMAN SITUATION

HD is a devastating disease with no cure. As discussed in this review, numerous therapeutic strategies, including drugs, growth factors and even cell grafting, have been tested in animal models of HD. No therapy that has so far been effective in animal models of HD has also shown significant effects in clinical trials in HD patients. Some of the animal studies have also given inconsistent results in the different models. Those differences could be due to particular features of the models used, different doses and routes of delivery, and different assessment protocols. Differences in efficacy of treatments may also depend upon the stages of the diseases at the time when the treatments are administered. Thus, prevention of disease onset might not require that the treatment interferes with the same pathogenetic mechanisms as if it is to cause slowing down of disease progression after the symptoms have already appeared. Regarding the assessment of efficacy, as summarized in Table 1, there is no general consensus on which assessment(s) should be used as measures of being "effective" to a treatment. Many trials use "length of survival" as a parameter to assess efficacy of treatment. In those studies, however, increased life spans are not necessarily accompanied by improved locomotor activity and motor coordination, which may reflect the quality of the life in human patients. In contrast, some trials use frequency of aggregates/inclusions as an endpoint of the treatments. Treated mice that displayed reduced formation of inclusions have often been found to exhibit longer survival and improvement in behavioral tests. However, there are also therapeutic studies in R6 mice where there was functional improvement, but no effect of aggregates/inclusions formation.

This questions the importance of the aggregates in the development of symptoms in R6 mice, and by extension even in HD patients.

The need for good animal models and that scientists study relevant parameters in these models cannot be overemphasized. Several transgenic and knock-in models of HD are available (for the knock-in models, see Menalled in this issue[164]), the most widely used one is the R6/2 mouse. Each mouse model is unique and can only partially mimic the HD phenotype as seen in humans. Obviously, due to the major differences in the normal behavioral repertoire of rodents and humans, major differences in symptoms between transgenic mice and patients with HD are to be expected. In addition, the neuropathological features seen in mice and man, such as aggregate formation, cell death, transmitter changes, alterations in neurogenesis, are at best analogous. It is important to know the detailed phenotypic features of a given mouse model before choosing it for a drug trial. Is the pathogenic feature that one is planning to study even present in the model? Can the drug be given at a time point when it is still possible to affect the pathogenic event? These issues may seem self-evident, but there are still many examples of where the experimental trial design has not taken them into account. For example, if a tested drug is supposed to affect huntingtin processing and cleavage, animals that only express N-terminal region of huntingtin, such as, the R6 lines, may not be optimal. Instead, transgenic or knock-in mice expressing full-length huntingtin may be better.[165,166] If inhibition of cell death is the main target, then examining cell numbers in the striatum and cortex of R6 mice seems to make little sense. Interestingly, the recent finding that orexin neurons gradually die in the lateral hypothalamus of the R6/2 mouse, and are lost in the end stage HD patients[26] means that we now have a novel and highly clinically relevant outcome parameter to study in therapeutic trials in R6/2 mice. The loss of orexin neurons is progressive, can easily be quantified, and is correlated with the appearance of narcoleptic sleep episodes. It will be interesting to see whether some of the drugs that have been reported to be beneficial in R6/2 mice also affect the survival of orexin neurons.

Acknowledgments: The work in the authors' lab is supported by the Swedish Research Council, the Hereditary Disease Foundation, the Swedish Society for Medicine, the Crafoord Foundation, the Hedlund Foundation and the Greta och Johan Kocks Foundation. N.P. is supported by the grant Biodegradable controlled drug delivery systems for the treatment of brain diseases (BCDDS) (QLRT-2000-02226); RTD-EU-project, Quality of Life and Management of Living Resources. The authors are members of The European Union concerted action consortium "Early pathogenetic markers for Slow Neurodegenerative Diseases" (EPSND, QLK6-CT-2000-00384) and would like to acknowledge the helpful discussions that have taken place within this network. We thank Ruben Smith, Joana Gil, Jorien van der Burg, and Diogo Ribeiro for critically reading this manuscript.

REFERENCES

1. Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* 87:493–506, 1996.
2. Menalled LB, Chesselet MF. Mouse models of Huntington's disease. *Trends Pharmacol Sci* 23:32–39, 2002.
3. Group HsDCR. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72:971–983, 1993.
4. Vonsattel JP, DiFiglia M. Huntington disease. *J Neuropathol Exp Neurol* 57:369–384, 1998.
5. Kremer HP, Roos RA, Dingjan GM, Bots GT, Bruyn GW, Hofman MA. The hypothalamic lateral tuberal nucleus and the characteristics of neuronal loss in Huntington's disease. *Neurosci Lett* 132:101–104, 1991.
6. Kremer HP, Roos RA, Dingjan G, Marani E, Bots GT. Atrophy of the hypothalamic lateral tuberal nucleus in Huntington's disease. *J Neuropathol Exp Neurol* 49:371–382, 1990.
7. Qin ZH, Wang Y, Sapp E, Cuiffo B, Wanker E, Hayden MR, et al. Huntingtin bodies sequester vesicle-associated proteins by a polyproline-dependent interaction. *J Neurosci* 24:269–281, 2004.
8. Landles C, Bates GP. Huntingtin and the molecular pathogenesis of Huntington's disease. *EMBO Rep* 5:958–963, 2004.
9. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 431:805–810, 2004.
10. Saudou F, Finkbeiner S, Devys D, Greenberg ME. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* 95:55–66, 1998.
11. Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, Singaraja R, et al. A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. *Neuron* 23:181–192, 1999.
12. Hockly E, Woodman B, Mahal A, Lewis CM, Bates G. Standardization and statistical approaches to therapeutic trials in the R6/2 mouse. *Brain Res Bull* 61:469–479, 2003.
13. Rubinsztein DC. Lessons from animal models of Huntington's disease. *Trends Genet* 18:202–209, 2002.
14. Luesse HG, Schiefer J, Spruenken A, Puls C, Block F, Kosinski CM. Evaluation of R6/2 HD transgenic mice for therapeutic studies in Huntington's disease: behavioral testing and impact of diabetes mellitus. *Behav Brain Res* 126:185–195, 2001.
15. Lione LA, Carter RJ, Hunt MJ, Bates GP, Morton AJ, Dunnett SB. Selective discrimination learning impairments in mice expressing the human Huntington's disease mutation. *J Neurosci* 19:10428–10437, 1999.
16. Murphy KP, Carter RJ, Lione LA, Mangiarini L, Mahal A, Bates GP, et al. Abnormal synaptic plasticity and impaired spatial cognition in mice transgenic for exon 1 of the human Huntington's disease mutation. *J Neurosci* 20:5115–5123, 2000.
17. Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, et al. Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. *J Neurosci* 19:3248–3257, 1999.
18. Hansson O, Guatteo E, Mercuri NB, Bernardi G, Li XJ, Castilho RF, et al. Resistance to NMDA toxicity correlates with appearance of nuclear inclusions, behavioural deficits and changes in calcium homeostasis in mice transgenic for exon 1 of the huntington gene. *Eur J Neurosci* 14:1492–1504, 2001.
19. Sathasivam K, Hobbs C, Turmaine M, Mangiarini L, Mahal A, Bertaux F, et al. Formation of polyglutamine inclusions in non-CNS tissue. *Hum Mol Genet* 8:813–822, 1999.
20. Hurlbert MS, Zhou W, Wasmeier C, Kaddis FG, Hutton JC, Freed CR. Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes. *Diabetes* 48:649–651, 1999.

21. Bjorkqvist M, Fex M, Renström E, Wierup N, Petersen A, Gil J, et al. The R6/2 transgenic mouse model of Huntington's disease develops diabetes due to deficient β-cell mass and altered exocytosis. *Hum Mol Genet* 14:565–574, 2005.
22. Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* 90:537–548, 1997.
23. Hansson O, Petersen A, Leist M, Nicotera P, Castilho RF, Brundin P. Transgenic mice expressing a Huntington's disease mutation are resistant to quinolinic acid-induced striatal excitotoxicity. *Proc Natl Acad Sci USA* 96:8727–8732, 1999.
24. Turmaine M, Raza A, Mahal A, Mangiarini L, Bates GP, Davies SW. Nonapoptotic neurodegeneration in a transgenic mouse model of Huntington's disease. *Proc Natl Acad Sci USA* 97: 8093–8097, 2000.
25. Iannicola C, Moreno S, Oliverio S, Nardacci R, Ciofi-Luzzatto A, Piacentini M. Early alterations in gene expression and cell morphology in a mouse model of Huntington's disease. *J Neurochem* 75:830–839, 2000.
26. Petersen A, Gil J, Maat-Schieman ML, Bjorkqvist M, Tanila H, Araujo IM, et al. Orexin loss in Huntington's disease. *Hum Mol Genet* 14:39–47, 2005.
27. Deleted in proof.
28. Klapstein GJ, Fisher RS, Zanjani H, Cepeda C, Jokel ES, Chesselet MF, et al. Electrophysiological and morphological changes in striatal spiny neurons in R6/2 Huntington's disease transgenic mice. *J Neurophysiol* 86:2667–2677, 2001.
29. Petersen A, Puschban Z, Lotharius J, NicNiocaill B, Wiekop P, O'Connor WT, et al. Evidence for dysfunction of the nigrostriatal pathway in the R6/1 line of transgenic Huntington's disease mice. *Neurobiol Dis* 11:134–146, 2002.
30. Morton AJ, Lagan MA, Skepper JN, Dunnett SB. Progressive formation of inclusions in the striatum and hippocampus of mice transgenic for the human Huntington's disease mutation. *J Neurocytol* 29:679–702, 2000.
31. Meade CA, Deng YP, Fusco FR, Del Mar N, Hersch S, Goldowitz D, et al. Cellular localization and development of neuronal intranuclear inclusions in striatal and cortical neurons in R6/2 transgenic mice. *J Comp Neurol* 449:241–269, 2002.
32. Kosinski CM, Cha JH, Young AB, Mangiarini L, Bates G, Schiefer J, et al. Intranuclear inclusions in subtypes of striatal neurons in Huntington's disease transgenic mice. *Neuroreport* 10:3891–3896, 1999.
33. Jana NR, Zemskov EA, Wang G, Nukina N. Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome c release. *Hum Mol Genet* 10:1049–1059, 2001.
34. Luthi-Carter R, Strand A, Peters NL, Solano SM, Hollingsworth ZR, Menon AS, et al. Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. *Hum Mol Genet* 9:1259–1271, 2000.
35. Luthi-Carter R, Hanson SA, Strand AD, Bergstrom DA, Chun W, Peters NL, et al. Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. *Hum Mol Genet* 11:1911–1926, 2002.
36. Bogdanov MB, Andreassen OA, Dedeoglu A, Ferrante RJ, Beal MF. Increased oxidative damage to DNA in a transgenic mouse model of Huntington's disease. *J Neurochem* 79:1246–1249, 2001.
37. Santamaria A, Perez-Severiano F, Rodriguez-Martinez E, Maldonado PD, Pedraza-Chaverri J, Rios C, et al. Comparative analysis of superoxide dismutase activity between acute pharmacological models and a transgenic mouse model of Huntington's disease. *Neurochem Res* 26:419–424, 2001.
38. Tabrizi SJ, Workman J, Hart PE, Mangiarini L, Mahal A, Bates G, et al. Mitochondrial dysfunction and free radical damage in the Huntington R6/2 transgenic mouse. *Ann Neurol* 47:80–86, 2000.
39. Perez-Severiano F, Escalante B, Vergara P, Rios C, Segovia J. Age-dependent changes in nitric oxide synthase activity and protein expression in striata of mice transgenic for the Huntington's disease mutation. *Brain Res* 951:36–42, 2002.
40. Deckel AW, Gordinier A, Nuttal D, Tang V, Kuwada C, Freitas R, et al. Reduced activity and protein expression of NOS in R6/2 HD transgenic mice: effects of L-NAME on symptom progression. *Brain Res* 919:70–81, 2001.
41. Deckel AW, Tang V, Nuttal D, Gary K, Elder R. Altered neuronal nitric oxide synthase expression contributes to disease progression in Huntington's disease transgenic mice. *Brain Res* 939:76–86, 2002.
42. Petersen A, Larsen KE, Behr GG, Romero N, Przedborski S, Brundin P, et al. Expanded CAG repeats in exon 1 of the Huntington's disease gene stimulate dopamine-mediated striatal neuron autophagy and degeneration. *Hum Mol Genet* 10:1243–1254, 2001.
43. Levine MS, Klapstein GJ, Koppel A, Gruen E, Cepeda C, Vargas ME, et al. Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. *J Neurosci Res* 58:515–532, 1999.
44. Cepeda C, Hurst RS, Calvert CR, Hernandez-Echeagaray E, Nguyen OK, Jocoy E, et al. Transient and progressive electrophysiological alterations in the corticostriatal pathway in a mouse model of Huntington's disease. *J Neurosci* 23:961–969, 2003.
45. Nicniocaill B, Haraldsson B, Hansson O, O'Connor WT, Brundin P. Altered striatal amino acid neurotransmitter release monitored using microdialysis in R6/1 Huntington transgenic mice. *Eur J Neurosci* 13:206–210, 2001.
46. Lievens JC, Woodman B, Mahal A, Spasic-Boscovic O, Samuel D, Kerkerian-Le Goff L, et al. Impaired glutamate uptake in the R6 Huntington's disease transgenic mice. *Neurobiol Dis* 8:807–821, 2001.
47. Behrens PF, Franz P, Woodman B, Lindenberg KS, Landwehrmeyer GB. Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. *Brain* 125:1908–1922, 2002.
48. Hickey MA, Reynolds GP, Morton AJ. The role of dopamine in motor symptoms in the R6/2 transgenic mouse model of Huntington's disease. *J Neurochem* 81:46–59, 2002.
49. Reynolds GP, Dalton CF, Tillery CL, Mangiarini L, Davies SW, Bates GP. Brain neurotransmitter deficits in mice transgenic for the Huntington's disease mutation. *J Neurochem* 72:1773–1776, 1999.
50. Ariano MA, Aronin N, Difiglia M, Tagle DA, Sibley DR, Leavitt BR, et al. Striatal neurochemical changes in transgenic models of Huntington's disease. *J Neurosci Res* 68:716–729, 2002.
51. Yohrling IG, Jiang GC, DeJohn MM, Robertson DJ, Vrana KE, Cha JH. Inhibition of tryptophan hydroxylase activity and decreased 5-HT1A receptor binding in a mouse model of Huntington's disease. *J Neurochem* 82:1416–1423, 2002.
52. Morton AJ, Faull RL, Edwardson JM. Abnormalities in the synaptic vesicle fusion machinery in Huntington's disease. *Brain Res Bull* 56:111–117, 2001.
53. Morton AJ, Edwardson JM. Progressive depletion of complexin II in a transgenic mouse model of Huntington's disease. *J Neurochem* 76:166–172, 2001.
54. Lievens JC, Woodman B, Mahal A, Bates GP. Abnormal phosphorylation of synapsin I predicts a neuronal transmission impairment in the R6/2 Huntington's disease transgenic mice. *Mol Cell Neurosci* 20:638–648, 2002.
55. Modregger J, DiProspero NA, Charles V, Tagle DA, Plomann M. PACSIN 1 interacts with huntingtin and is absent from synaptic varicosities in presymptomatic Huntington's disease brains. *Hum Mol Genet* 11:2547–2558, 2002.
56. Smith R, Petersen A, Bates G, Brundin P, Li JY. Depletion of rabphilin 3A in a transgenic mouse model (R6/1) of Huntington's Disease, a possible culprit in synaptic dysfunction. *Neurobiol Dis*, in press.
57. Cha JH, Frey AS, Alsdorf SA, Kerner JA, Kosinski CM, Mangiarini L, et al. Altered neurotransmitter receptor expression in transgenic mouse models of Huntington's disease. *Philos Trans R Soc Lond B Biol Sci* 354:981–989, 1999.
58. Bibb JA, Yan Z, Svenningsson P, Snyder GL, Pieribone VA, Horiuchi A, et al. Severe deficiencies in dopamine signaling in presymptomatic Huntington's disease mice. *Proc Natl Acad Sci USA* 97:6809–6814, 2000.

59. van Dellen A, Blakemore C, Deacon R, York D, Hannan AJ. Delaying the onset of Huntington's in mice. *Nature* 404:721–722, 2000.
60. Zucker B, Luthi-Carter R, Kama JA, Dunah AW, Stern EA, Fox JH, et al. Transcriptional dysregulation in striatal projection- and interneurons in a mouse model of Huntington's disease: neuronal selectivity and potential neuroprotective role of HAP1. *Hum Mol Genet* 14:179–189, 2005.
61. Petersen A, Hansson O, Puschban Z, Sapp E, Romero N, Castilho RF, et al. Mice transgenic for exon 1 of the Huntington's disease gene display reduced striatal sensitivity to neurotoxicity induced by dopamine and 6-hydroxydopamine. *Eur J Neurosci* 14:1425–1435, 2001.
62. Hansson O, Castilho RF, Korhonen L, Lindholm D, Bates GP, Brundin P. Partial resistance to malonate-induced striatal cell death in transgenic mouse models of Huntington's disease is dependent on age and CAG repeat length. *J Neurochem* 78:694–703, 2001.
63. Schiefer J, Alberty A, Dose T, Oliva S, Noth J, Kosinski CM. Huntington's disease transgenic mice are resistant to global cerebral ischemia. *Neurosci Lett* 334:99–102, 2002.
64. Morton AJ, Leavens W. Mice transgenic for the human Huntington's disease mutation have reduced sensitivity to kainic acid toxicity. *Brain Res Bull* 52:51–59, 2000.
65. Hickey MA, Morton AJ. Mice transgenic for the Huntington's disease mutation are resistant to chronic 3-nitropropionic acid-induced striatal toxicity. *J Neurochem* 75:2163–2171, 2000.
66. Li JY, Plomann M, Brundin P. Huntington's disease: a synaptopathy? *Trends Mol Med* 9:414–420, 2003.
67. Rubinsztein DC, Carmichael J. Huntington's disease: molecular basis of neurodegeneration. *Expert Rev Mol Med* 5:1–21, 2003.
68. Sugars KL, Rubinsztein DC. Transcriptional abnormalities in Huntington disease. *Trends Genet* 19:233–238, 2003.
69. Ciechanover A, Brundin P. The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. *Neuron* 40:427–446, 2003.
70. Beal MF. Energetics in the pathogenesis of neurodegenerative diseases. *Trends Neurosci* 23:298–304, 2000.
71. Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* 292:1552–1555, 2001.
72. Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, Zoghbi HY. Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nat Genet* 19:148–154, 1998.
73. Fink AL. Chaperone-mediated protein folding. *Physiol Rev* 79: 425–449, 1999.
74. Hartl FU, Hayer-Hartl M. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295:1852–1858, 2002.
75. Hay DG, Sathasivam K, Tobaben S, Stahl B, Marber M, Mestril R, et al. Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. *Hum Mol Genet* 13:1389–1405, 2004.
76. Hansson O, Nylandsted J, Castilho RF, Leist M, Jaattela M, Brundin P. Overexpression of heat shock protein 70 in R6/2 Huntington's disease mice has only modest effects on disease progression. *Brain Res* 970:47–57, 2003.
77. Sittler A, Lurz R, Lueder G, Priller J, Hayer-Hartl MK, Hartl FU, et al. Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum Mol Genet* 10:1307–1315, 2001.
78. Smith DL, Portier R, Woodman B, Hockly E, Mahal A, Klunk WE, et al. Inhibition of polyglutamine aggregation in R6/2 HD brain slices-complex dose-response profiles. *Neurobiol Dis* 8:1017–1026, 2001.
79. Heiser V, Scherzinger E, Boeddrich A, Nordhoff E, Lurz R, Schugardt N, et al. Inhibition of huntingtin fibrillogenesis by specific antibodies and small molecules: implications for Huntington's disease therapy. *Proc Natl Acad Sci USA* 97:6739–6744, 2000.
80. Sanchez I, Mahlke C, Yuan J. Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. *Nature* 421:373–379, 2003.
81. Yamamoto A, Lucas JJ, Hen R. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell* 101:57–66, 2000.
82. Heiser V, Engemann S, Brocker W, Dunkel I, Boeddrich A, Waelter S, et al. Identification of benzothiazoles as potential polyglutamine aggregation inhibitors of Huntington's disease by using an automated filter retardation assay. *Proc Natl Acad Sci USA* 99 [Suppl 4]:16400–16406, 2002.
83. Schiefer J, Landwehrmeyer GB, Luesse HG, Sprunken A, Puls C, Milkereit A, et al. Riluzole prolongs survival time and alters nuclear inclusion formation in a transgenic mouse model of Huntington's disease. *Mov Disord* 17:748–757, 2002.
84. Tanaka M, Machida Y, Niu S, Ikeda T, Jana NR, Doi H, et al. Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat Med* 10:148–154, 2004.
85. Kegel KB, Meloni AR, Yi Y, Kim YJ, Doyle E, Cuiffo BG, et al. Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. *J Biol Chem* 277:7466–7476, 2002.
86. Martindale D, Hackam A, Wieczorek A, Ellerby L, Wellington C, McCutcheon K, et al. Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nat Genet* 18:150–154, 1998.
87. Preisinger E, Jordan BM, Kazantsev A, Housman D. Evidence for a recruitment and sequestration mechanism in Huntington's disease. *Philos Trans R Soc Lond B Biol Sci* 354:1029–1034, 1999.
88. Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL, et al. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. *Nature* 413:739–743, 2001.
89. Hockly E, Richon VM, Woodman B, Smith DL, Zhou X, Rosa E, et al. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. *Proc Natl Acad Sci USA* 100:2041–2046, 2003.
90. Ferrante RJ, Kubilus JK, Lee J, Ryu H, Beesen A, Zucker B, et al. Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. *J Neurosci* 23:9418–9427, 2003.
91. Ferrante RJ, Ryu H, Kubilus JK, D'Mello S, Sugars KL, Lee J, et al. Chemotherapy for the brain: the antitumor antibiotic mithramycin prolongs survival in a mouse model of Huntington's disease. *J Neurosci* 24:10335–10342, 2004.
92. Kahlem P, Green H, Djian P. Transglutaminase action imitates Huntington's disease: selective polymerization of Huntingtin containing expanded polyglutamine. *Mol Cell* 1:595–601, 1998.
93. Karpuj MV, Garren H, Slunt H, Price DL, Gusella J, Becher MW, et al. Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. *Proc Natl Acad Sci USA* 96:7388–7393, 1999.
94. Lesort M, Chun W, Johnson GV, Ferrante RJ. Tissue transglutaminase is increased in Huntington's disease brain. *J Neurochem* 73:2018–2027, 1999.
95. Karpuj MV, Becher MW, Springer JE, Chabas D, Youssef S, Pedotti R, et al. Prolonged survival and decreased abnormal movements in transgenic model of Huntington disease, with administration of the transglutaminase inhibitor cystamine. *Nat Med* 8:143–149, 2002.
96. Dedeoglu A, Kubilus JK, Jeitner TM, Matson SA, Bogdanov M, Kowall NW, et al. Therapeutic effects of cystamine in a murine model of Huntington's disease. *J Neurosci* 22:8942–8950, 2002.
97. Fox JH, Barber DS, Singh B, Zucker B, Swindell MK, Norflus F, et al. Cystamine increases L-cysteine levels in Huntington's disease transgenic mouse brain and in a PC12 model of polyglutamine aggregation. *J Neurochem* 91:413–422, 2004.
98. Goldberg YP, Nicholson DW, Rasper DM, Kalchman MA, Koide HB, Graham RK, et al. Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nat Genet* 13:442–449, 1996.
99. Wellington CL, Ellerby LM, Gutekunst CA, Rogers D, Warby S, Graham RK, et al. Caspase cleavage of mutant huntingtin pre-

cedes neurodegeneration in Huntington's disease. *J Neurosci* 22: 7862–7872, 2002.
100. Gafni J, Hermel E, Young JE, Wellington CL, Hayden MR, Ellerby LM. Inhibition of calpain cleavage of huntingtin reduces toxicity: accumulation of calpain/caspase fragments in the nucleus. *J Biol Chem* 279:20211–20220, 2004.
101. Lunkes A, Lindenberg KS, Ben-Haiem L, Weber C, Devys D, Landwehrmeyer GB, et al. Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. *Mol Cell* 10:259–269, 2002.
102. Gafni J, Ellerby LM. Calpain activation in Huntington's disease. *J Neurosci* 22:4842–4849, 2002.
103. Hermel E, Gafni J, Propp SS, Leavitt BR, Wellington CL, Young JE, et al. Specific caspase interactions and amplification are involved in selective neuronal vulnerability in Huntington's disease. *Cell Death Differ* 11:424–438, 2004.
104. Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, et al. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med* 6:797–801, 2000.
105. Wang X, Zhu S, Drozda M, Zhang W, Stavrovskaya IG, Cattaneo E, et al. Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. *Proc Natl Acad Sci USA* 100:10483–10487, 2003.
106. Smith DL, Woodman B, Mahal A, Sathasivam K, Ghazi-Noori S, Lowden PA, et al. Minocycline and doxycycline are not beneficial in a model of Huntington's disease. *Ann Neurol* 54:186–196, 2003.
107. Denovan-Wright EM, Devarajan S, Dursun SM, Robertson HA. Maintained improvement with minocycline of a patient with advanced Huntington's disease. *J Psychopharmacol* 16:393–394, 2002.
108. Bonelli RM, Hodl AK, Hofmann P, Kapfhammer HP. Neuroprotection in Huntington's disease: a 2-year study on minocycline. *Int Clin Psychopharmacol* 19:337–342, 2004.
109. Thomas M, Ashizawa T, Jankovic J. Minocycline in Huntington's disease: a pilot study. *Mov Disord* 19:692–695, 2004.
110. Group HR. Minocycline safety and tolerability in Huntington disease. *Neurology* 63:547–549, 2004.
111. Kiechle T, Dedeoglu A, Kubilus J, Kowall NW, Beal MF, Friedlander RM, et al. Cytochrome C and caspase-9 expression in Huntington's disease. *Neuromolecular Med* 1:183–195, 2002.
112. Sanchez I, Xu CJ, Juo P, Kakizaka A, Blenis J, Yuan J. Caspase-8 is required for cell death induced by expanded polyglutamine repeats. *Neuron* 22:623–633, 1999.
113. Ona VO, Li M, Vonsattel JP, Andrews LJ, Khan SQ, Chung WM, et al. Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* 399:263–267, 1999.
114. Friedlander RM. Apoptosis and caspases in neurodegenerative diseases. *N Engl J Med* 348:1365–1375, 2003.
115. Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 117:561–574, 2004.
116. Zhang Y, Ona VO, Li M, Drozda M, Dubois-Dauphin M, Przedborski S, et al. Sequential activation of individual caspases, and of alterations in Bcl-2 proapoptotic signals in a mouse model of Huntington's disease. *J Neurochem* 87:1184–1192, 2003.
117. Choo YS, Johnson GV, MacDonald M, Detloff PJ, Lesort M. Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. Hum Mol Genet 2004.
118. Koroshetz WJ, Jenkins BG, Rosen BR, Beal MF. Energy metabolism defects in Huntington's disease and effects of coenzyme Q10. *Ann Neurol* 41:160–165, 1997.
119. Ferrante RJ, Andreassen OA, Dedeoglu A, Ferrante KL, Jenkins BG, Hersch SM, et al. Therapeutic effects of coenzyme Q10 and remacemide in transgenic mouse models of Huntington's disease. *J Neurosci* 22:1592–1599, 2002.
120. Schilling G, Coonfield ML, Ross CA, Borchelt DR. Coenzyme Q10 and remacemide hydrochloride ameliorate motor deficits in a Huntington's disease transgenic mouse model. *Neurosci Lett* 315:149–153, 2001.
121. Group HS. A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. *Neurology* 57: 397–404, 2001.
122. Beal MF. Huntington's disease, energy, and excitotoxicity. *Neurobiol Aging* 15:275–276, 1994.
123. Cha JH, Kosinski CM, Kerner JA, Alsdorf SA, Mangiarini L, Davies SW, et al. Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an abnormal human huntington disease gene. *Proc Natl Acad Sci USA* 95:6480–6485, 1998.
124. Cepeda C, Ariano MA, Calvert CR, Flores-Hernandez J, Chandler SH, Leavitt BR, et al. NMDA receptor function in mouse models of Huntington disease. *J Neurosci Res* 66:525–539, 2001.
125. Schiefer J, Sprunken A, Puls C, Luesse HG, Milkereit A, Milkereit E, et al. The metabotropic glutamate receptor 5 antagonist MPEP and the mGluR2 agonist LY379268 modify disease progression in a transgenic mouse model of Huntington's disease. *Brain Res* 1019:246–254, 2004.
126. Ferrante RJ, Andreassen OA, Jenkins BG, Dedeoglu A, Kuemmerle S, Kubilus JK, et al. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J Neurosci* 20:4389–4397, 2000.
127. Andreassen OA, Dedeoglu A, Ferrante RJ, Jenkins BG, Ferrante KL, Thomas M, et al. Creatine increase survival and delays motor symptoms in a transgenic animal model of Huntington's disease. *Neurobiol Dis* 8:479–491, 2001.
128. Dedeoglu A, Kubilus JK, Yang L, Ferrante KL, Hersch SM, Beal MF, et al. Creatine therapy provides neuroprotection after onset of clinical symptoms in Huntington's disease transgenic mice. *J Neurochem* 85:1359–1367, 2003.
129. Verbessem P, Lemiere J, Eijnde BO, Swinnen S, Vanhees L, Van Leemputte M, et al. Creatine supplementation in Huntington's disease: a placebo-controlled pilot trial. *Neurology* 61:925–930, 2003.
130. Perez-Severiano F, Rios C, Segovia J. Striatal oxidative damage parallels the expression of a neurological phenotype in mice transgenic for the mutation of Huntington's disease. *Brain Res* 862:234–237, 2000.
131. Clifford JJ, Drago J, Natoli AL, Wong JY, Kinsella A, Waddington JL, et al. Essential fatty acids given from conception prevent topographies of motor deficit in a transgenic model of Huntington's disease. *Neuroscience* 109:81–88, 2002.
132. Das UN, Vaddadi KS. Essential fatty acids in Huntington's disease. *Nutrition* 20:942–947, 2004.
133. Vaddadi K. Dyskinesias and their treatment with essential fatty acids: a review. *Prostaglandins Leukot Essent Fatty Acids* 55: 89–94, 1996.
134. Vaddadi KS, Soosai E, Chiu E, Dingjan P. A randomised, placebo-controlled, double blind study of treatment of Huntington's disease with unsaturated fatty acids. *Neuroreport* 13:29–33, 2002.
135. Norflus F, Nanje A, Gutekunst CA, Shi G, Cohen J, Bejarano M, et al. Anti-inflammatory treatment with acetylsalicylate or rofecoxib is not neuroprotective in Huntington's disease transgenic mice. *Neurobiol Dis* 17:319–325, 2004.
136. Klivenyi P, Ferrante RJ, Gardian G, Browne S, Chabrier PE, Beal MF. Increased survival and neuroprotective effects of BN82451 in a transgenic mouse model of Huntington's disease. *J Neurochem* 86:267–272, 2003.
137. Rodrigues CM, Stieers CL, Keene CD, Ma X, Kren BT, Low WC, et al. Tauroursodeoxycholic acid partially prevents apoptosis induced by 3-nitropropionic acid: evidence for a mitochondrial pathway independent of the permeability transition. *J Neurochem* 75:2368–2379, 2000.
138. Rodrigues CM, Sola S, Brito MA, Brondino CD, Brites D, Moura JJ. Amyloid β-peptide disrupts mitochondrial membrane lipid and protein structure: protective role of tauroursodeoxycholate. *Biochem Biophys Res Commun* 281:468–474, 2001.
139. Keene CD, Rodrigues CM, Eich T, Chhabra MS, Steer CJ, Low WC. Tauroursodeoxycholic acid, a bile acid, is neuroprotective in a transgenic animal model of Huntington's disease. *Proc Natl Acad Sci USA* 99:10671–10676, 2002.
140. Carmichael J, Sugars KL, Bao YP, Rubinsztein DC. Glycogen

synthase kinase-3β inhibitors prevent cellular polyglutamine toxicity caused by the Huntington's disease mutation. *J Biol Chem* 277:33791–33798, 2002.
141. Wood NI, Morton AJ. Chronic lithium chloride treatment has variable effects on motor behaviour and survival of mice transgenic for the Huntington's disease mutation. *Brain Res Bull* 61: 375–383, 2003.
142. Rebec GV, Barton SJ, Ennis MD. Dysregulation of ascorbate release in the striatum of behaving mice expressing the Huntington's disease gene. *J Neurosci* 22:RC202, 2002.
143. Rebec GV, Barton SJ, Marseilles AM, Collins K. Ascorbate treatment attenuates the Huntington behavioral phenotype in mice. *Neuroreport* 14:1263–1265, 2003.
144. Alberch J, Perez-Navarro E, Canals JM. Neurotrophic factors in Huntington's disease. *Prog Brain Res* 146:195–229, 2004.
145. Popovic N, Maingay M, Kirik D, Brundin P. Lentiviral gene delivery of GDNF into the striatum of R6/2 Huntington mice fails to attenuate behavioral and neuropathological changes. *Exp Neurol* 193:65–74, 2005.
146. Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 293:493–498, 2001.
147. Baquet ZC, Gorski JA, Jones KR. Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. *J Neurosci* 24:4250–4258, 2004.
148. Canals JM, Pineda JR, Torres-Peraza JF, Bosch M, Martin-Ibanez R, Munoz MT, et al. Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. *J Neurosci* 24:7727–7739, 2004.
149. Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H, Cordelieres FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell* 118:127–138, 2004.
150. Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, et al. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nat Genet* 35: 76–83, 2003.
151. Ferrer I, Goutan E, Marin C, Rey MJ, Ribalta T. Brain-derived neurotrophic factor in Huntington disease. *Brain Res* 866:257–261, 2000.
152. Cepeda C, Starling AJ, Wu N, Nguyen OK, Uzgil B, Soda T, et al. Increased GABAergic function in mouse models of Huntington's disease: reversal by BDNF. *J Neurosci Res* 78:855–867, 2004.
153. van Dellen A, Deacon R, York D, Blakemore C, Hannan AJ. Anterior cingulate cortical transplantation in transgenic Huntington's disease mice. *Brain Res Bull* 56:313–318, 2001.
154. Dunnett SB, Carter RJ, Watts C, Torres EM, Mahal A, Mangiarini L, et al. Striatal transplantation in a transgenic mouse model of Huntington's disease. *Exp Neurol* 154:31–40, 1998.
155. Carter RJ, Hunt MJ, Morton AJ. Environmental stimulation increases survival in mice transgenic for exon 1 of the Huntington's disease gene. *Mov Disord* 15:925–937, 2000.
156. Hockly E, Cordery PM, Woodman B, Mahal A, van Dellen A, Blakemore C, et al. Environmental enrichment slows disease progression in R6/2 Huntington's disease mice. *Ann Neurol* 51: 235–242, 2002.
157. Glass M, van Dellen A, Blakemore C, Hannan AJ, Faull RL. Delayed onset of huntington's disease in mice in an enriched environment correlates with delayed loss of cannabinoid CB1 receptors. *Neuroscience* 123:207–212, 2004.
158. Schilling G, Savonenko AV, Coonfield ML, Morton JL, Vorovich E, Gale A, et al. Environmental, pharmacological, and genetic modulation of the HD phenotype in transgenic mice. *Exp Neurol* 187:137–149, 2004.
159. Spires TL, Grote HE, Varshney NK, Cordery PM, van Dellen A, Blakemore C, et al. Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. *J Neurosci* 24:2270–2276, 2004.
160. Curtis MA, Penney EB, Pearson AG, van Roon-Mom WM, Butterworth NJ, Dragunow M, et al. Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. *Proc Natl Acad Sci USA* 100:9023–9027, 2003.
161. Gil JM, Leist M, Popovic N, Brundin P, Petersen A. Asialoerythropoietin is not effective in the R6/2 line of Huntington's disease mice. *BMC Neurosci* 5:17, 2004.
162. Lazic SE, Grote H, Armstrong RJ, Blakemore C, Hannan AJ, van Dellen A, et al. Decreased hippocampal cell proliferation in R6/1 Huntington's mice. *Neuroreport* 15:811–813, 2004.
163. Erbayraktar S, Grasso G, Sfacteria A, Xie QW, Coleman T, Kreilgaard M, et al. Asialoerythropoietin is a nonerythropoietic cytokine with broad neuroprotective activity in vivo. *Proc Natl Acad Sci USA* 100:6741–6746, 2003.
164. Menalled LB. Knock-in mouse models of huntington's disease. *NeuroRx* 2:465–470, 2005.
165. Slow EJ, Van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, et al. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. *Hum Mol Genet* 12:1555–1567, 2003.
166. Lin CH, Tallaksen-Greene S, Chien WM, Cearley JA, Jackson WS, Crouse AB, et al. Neurological abnormalities in a knock-in mouse model of Huntington's disease. *Hum Mol Genet* 10:137–144, 2001.